11.0 PRACTICAL CONSIDERATIONS

Several issues are taken into account when assessing the practicality of using an *in vitro* test method in place of an *in vivo* test method. In addition to reliability and accuracy evaluations, assessments of the equipment and supplies needed for the *in vitro* test method, level of personnel training, costs of the *in vitro* test method, and time to complete the method are necessary. This information provides additional information as whether the time, personnel cost, and effort required to conduct the test method is considered reasonable.

11.1 Transferability of the HET-CAM Test Method

Test method transferability is defined as the ability of a test method to be accurately and reliably performed by different, competent laboratories (ICCVAM 2003). Issues of transferability include laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. The degree of transferability of a test method affects its interlaboratory reproducibility.

11.1.1 Facilities and Major Fixed Equipment Required to Conduct a Study

The facilities needed to conduct the HET-CAM test method are widely available, and the necessary equipment is readily available from major suppliers. Major facilities necessary for performing the HET-CAM test method are standard toxicology, biochemistry, or molecular biology laboratory supplies. There are no specific requirements regarding the facility at which the test is conducted (e.g., sterile environment). However, it would seem appropriate to conduct the assay under ambient temperature and humidity conditions. To perform the test method an incubator that can rotate samples is needed. Depending upon the features of such an incubator, the cost of obtaining such an incubator through scientific vendors (e.g. Fisher Scientific, Phoenix Equipment Company) can range from about $130 to $4000.

The *in vivo* ocular toxicity test method requires that facilities develop and maintain an animal facility that adheres to pertinent State and Federal regulations. Personnel that are trained and skilled in dealing with such facilities also are needed for the *in vivo* test method. These facilities or personnel are not needed to conduct the HET-CAM test method. Similar to the *in vitro* test method, the *in vivo* test method uses equipment that is readily available from major suppliers.

11.1.2 General Availability of Other Necessary Equipment and Supplies

All other necessary equipment and supplies (e.g., candling light, rotating saw blade, pipettes, flasks, stop watch) are readily available from major scientific supply vendors.

11.2 Training Considerations

Training considerations are defined as the level of instruction needed for personnel to conduct the test methods accurately and reliably (ICCVAM 2003). Evaluation of the level of training and expertise needed to conduct the test method reliably and accurately, as well as the training requirements needed to ensure that personnel are competent in the test method, are discussed below.

11.2.1 Required Level of Training and Expertise Needed To Conduct the HET-CAM Test Method

An assessment of the protocols described in the reports reviewed in this BRD indicates that basic laboratory skills and training in embryo handling are necessary to conduct the HET-CAM test method. Some specialized training in removing the eggshell without damaging the inner membrane of the egg and removing the inner membrane from the CAM may be necessary to ensure competency in preparing the egg for the test method. Additionally, training in identifying the development of each of the three evaluated endpoints is necessary. A training video or other visual media to provide guidance on development of endpoints may be considered for use.

The level of training needed for the HET-CAM test method is not significantly greater than that required to conduct the *in vivo* ocular toxicity test method. Both the *in vivo* and *in vitro* test methods require developing competence in identifying endpoint development.

11.2.2 Training Requirements Needed to Demonstrate Proficiency

There are currently no known proficiency criteria used to ensure that personnel who are performing the test method are competent. Rather, this must be demonstrated through experience with the oversight of an experienced supervisor. Once the technician has demonstrated competence in identifying the study endpoints, it would seem appropriate for routine assessments of observations among trained personnel be conducted to ensure consistency.

11.3 Cost Considerations

A GLP-compliant EPA OPPTS Series 870 Acute Eye Irritation test (EPA 1998) or OECD TG 405 test (OECD 2002) at MB Research Laboratories (Spinnerstown, PA) ranges from $765 for a three day/three animal study up to $1665 for a 21 day/three animal study (MB Research laboratories, personal communication). The current costs of performing a GLP-compliant HET-CAM test have not yet been identified but are expected to be equivalent to or lower than the cost of an *in vivo* rabbit eye test.

11.4 Time Considerations

Use of the HET-CAM test method would significantly reduce the time needed to assess the ability of a test substance to induce ocular corrosivity or severe irritancy, when compared to the currently accepted *in vivo* rabbit eye test method. The *in vivo* Draize rabbit eye test is typically carried out for a minimum of one hour to three days. Depending upon the severity of ocular effects produced by a test substance, the method can be extended for up to 21 days to assess reversibility of observed effects. Completion of the HET-CAM test method requires a nine-day pre-treatment incubation period, followed by approximately one hour for the treatment and observation/measurement period.